AMINO ACID SENSITIVITY OF THE DACTYL CHEMORECEPTORS OF CARCINIDES MAENAS[1]

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In contrast with the present advanced state of knowledge concerning the physiology of insect chemoreceptors the remainder of the Arthropoda are essentially unknown in this regard excepting electrophysiological observations on the xiphosuran, *Limulus* (Barber, 1956, 1961), and several fresh-water and terrestrial forms (Hodgson, 1958). Although behavioral studies have demonstrated a well developed contact chemical sense generally distributed over the integument in marine crustaceans (Luther, 1930), a physiological analysis of its sensory basis has yet to be reported. Such an analysis is likely to be of more than passing interest since, considering the diet of crustaceans, their chemoreceptors are likely to be responsive to proteins and amino acids. They may consequently be expected to present an array of relationships between molecular configuration and receptor activation unlike any which have been well studied in arthropods.

In this report we describe a simple electrophysiological preparation of crustacean chemoreceptors and present evidence concerning their responses to a number of substances, principally amino acids. A preliminary summary of this work has been given (Case, Gwilliam and Hanson, 1960).

Materials and Methods

While only first walking leg dactyls of male *Carcinides maenas* were used in these experiments, chemoreceptor activity was readily demonstrable in the leg nerves of various other decapods including *Libinia emarginata, Callinectes sapidus,* and *Paguris pollicaris.*

The dactyl was prepared for electrical recording by removing all proximal segments and leaving intact approximately a 1-cm. length of nerve consisting at this level of two prominent bundles which both possess chemoreceptor fibers. A bridge of wax was used to support the dactyl with its tip in air and the nerve immersed in sea water. Small bundles of nerve fibers were dissected and arranged on bare platinum electrodes for recording in air or mineral oil, with a recording system consisting of a Grass P5 A.C. amplifier and Tektronix 502 oscilloscope. A loudspeaker monitor was an extremely useful adjunct to photographic recording.

Chemical stimuli were applied to the dactyl as uniform-sized drops of stimulant in filtered, autoclaved sea water. Immediately before application of a test stimulus the dactyl tip was exposed to several rapidly applied drops of sea water in order to

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adapt mechanoreceptors as much as possible. During all experiments the preparation was frequently tested with *Mytilus* extract (prepared by grinding the soft parts of a *Mytilus* in about 25 ml. of sea water) or .001 to .005 *M* l-glutamic acid and discarded once responses to these fell below initial magnitudes. Ordinarily, deterioration of the preparation presented little difficulty. With reasonable care a chemoreceptor bundle remained in satisfactory condition for an hour or longer.

Responses of the dactyl chemoreceptors to test substances were evaluated by comparing the activity induced by a drop of the test substance with the effect of a drop of .001 *M* l-glutamic acid. Data were evaluated from film records and by direct aural and visual comparisons of oscilloscopic activity. For purposes of tabulation these responses were recorded on a scale of 4 in which 0 was equal to a response indistinguishable from the effect of a drop of sea water and 4 was equal to the effect of .001 *M* l-glutamic acid.

Results

1. *General*

In nearly all preparations many nerve fibers could be isolated which mediated responses to *Mytilus* extract. Except in rare instances these were difficult to isolate

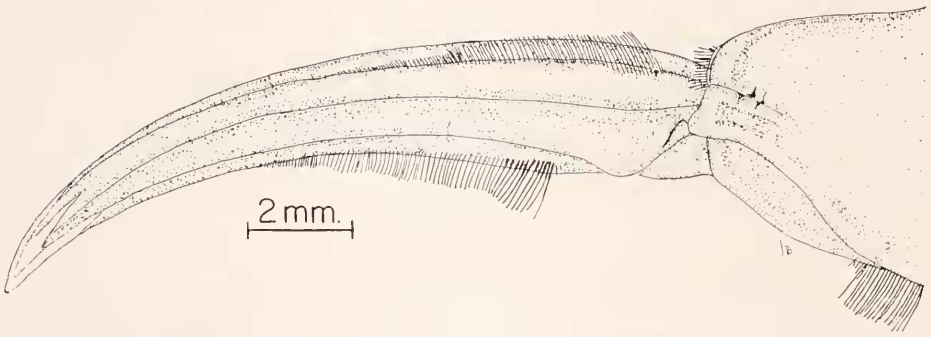

Figure 1. Dactyl of first walking leg of *Carcinides maenas*.

from larger fibers carrying mechanoreceptor impulses. Indeed, there is every possibility that at least some of the chemoreceptor units themselves may respond to mechanical stimulation, although it is clear from our data that not all do. In practice, the nearly unavoidable mixing of the two types of activity was not particularly bothersome since the mechanoreceptor responses were of short latency and adapted rapidly, while the slowly adapting chemoreceptor units had considerably longer latency, ranging from 15 to 250 mscs., measured from the first mechanoreceptor spike and varying inversely with stimulant concentration.

The nature of the sensory endings mediating chemoreceptor activity in the dactyl remains unknown. Application of sufficiently localized stimuli proved so difficult that about all that can be said is that chemoreceptor activity originates from the distal one-third of the dactyl as well as from more proximal regions. While this does not eliminate a chemoreceptive role for the long mechanoreceptor hairs of the proximal two-thirds of the dactyl (Fig. 1), it makes certain that other chemoreceptors must be present. These may be the Büschelorganen of Luther (1930) which

TABLE I

Response thresholds of Carcinides dactyl chemoreceptors

Substance	Number of dactyls tested	Intensity of response	Concentration (*M*)
A. Glutamic acid and related substances:			
l-glutamic acid[1]	18	3	5.0×10^{-5}
d-glutamic acid[6]	7	1	5.0×10^{-4}
		0	5.0×10^{-5}
glycyl-l-glutamic acid[1]	6	1	4.0×10^{-4}
		0	4.0×10^{-5}
l-glutamine[2]	12	1	5.0×10^{-4}
		0	5.0×10^{-5}
n-acetyl-l-glutamic[5]	7	1	1.0×10^{-3}
		0	1.0×10^{-5}
reduced glutathione[1]	7	1	5.0×10^{-3}
		0	5.0×10^{-4}
α-methyl glutamic acid[5]	3	1	3.0×10^{-2}
		0	1.0×10^{-3}
B. Other amino acids:			
glycine[1]	6	1	1.0×10^{-4}
		0	1.0×10^{-5}
l-ornithine[5]	3	1	1.0×10^{-2}
		0	1.0×10^{-3}
d-aspartic acid[6]	6	2	1.2×10^{-2}
l-aspartic acid[1]	6	1	1.2×10^{-2}
d-leucine[6]	7	2	2.5×10^{-2}
l-leucine[6]	7	1	2.5×10^{-2}
l-proline[2]	6	2	5.0×10^{-2}
		0	2.5×10^{-2}
γ-amino butyric acid[1]	5	1	0.2
		0	0.1
C. Organic acids:			
glutaric acid[4]	8	2	3.0×10^{-2}
		0	1.0×10^{-3}
α-keto glutaric acid[1]	5	2	5.0×10^{-2}
succinic acid[3]	6	2	1.0×10^{-3}
		0	1.0×10^{-4}
acetic acid[3]	2	1	0.1
D. Miscellaneous			
sucrose[3]	3	1	0.25
lactose[3]	3	0	0.25
methanol[3]	2	1	0.10
n-propanol[3]	2	1	0.10
n-butanol[3]	3	1	0.50
sea water	2	1	150%

Sources of chemicals: [1] Nutritional Biochemicals Corp.; [2] Matheson, Coleman, and Bell; [3] Fisher Scientific Corp.; [4] Eastman; [5] Sigma Chemical Company; [6] Mann Research Laboratories; [7] Merck and Company.

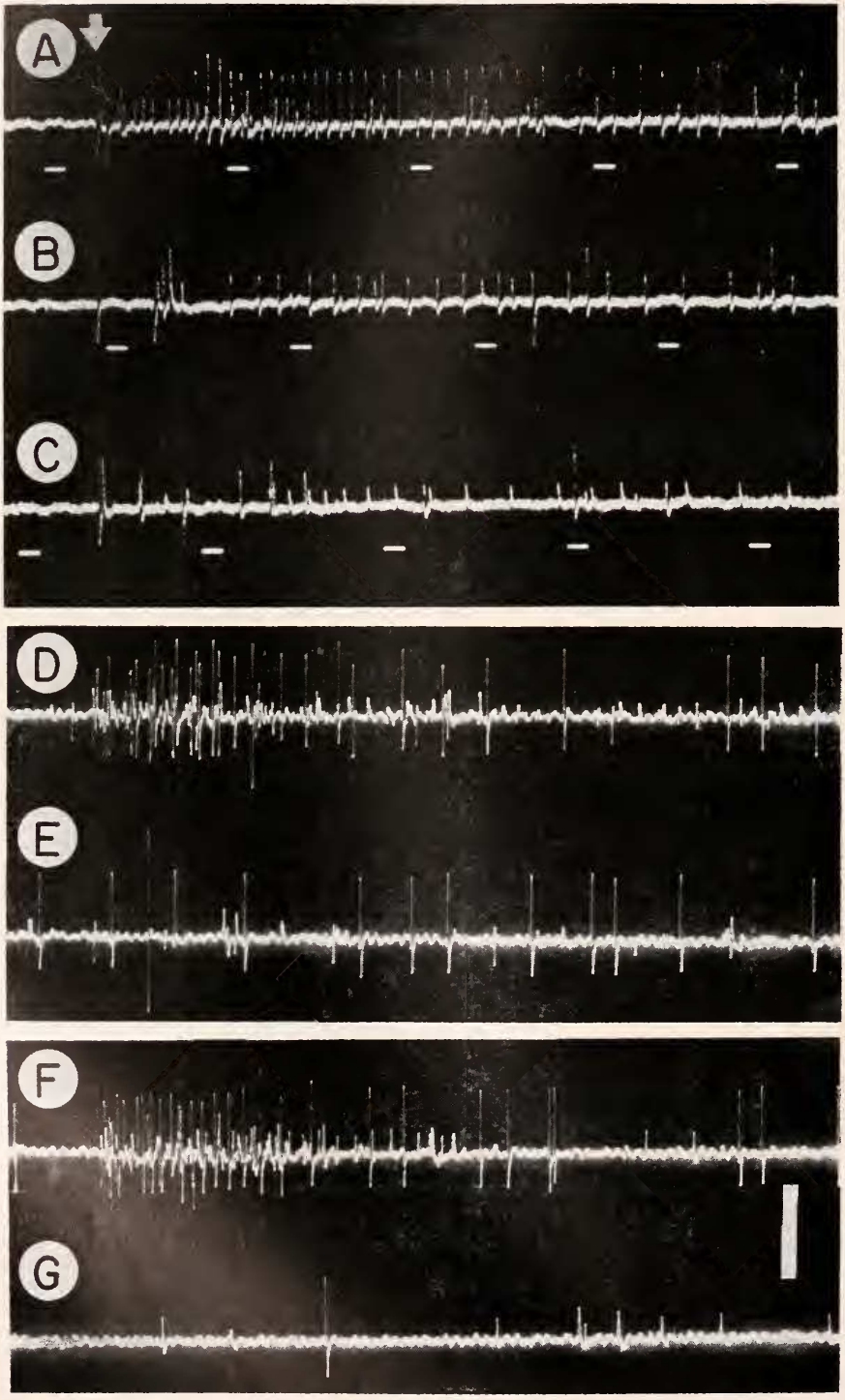

FIGURE 2.

we have also observed extending to the dactyl tip, especially in the grooves of the heavily chitinized cap. Both these and the mechanoreceptor hairs have been shown to be permeable to aqueous solutions of crystal violet in preparations for which we are indebted to Dr. E. H. Slifer, who finds the presence of such permeability in insects to be characteristic of chemoreceptive endings (Slifer, 1960).

2. *Receptor specificity*

In considering these data it must be remembered that our experimental technique has confined the study to a selected category, namely those sensory units which are responsive both to *Mytilus* extract and dilute glutamic acid. It may therefore be presumed that these observations on specificity are not applicable to the total chemoreceptor population of the dactyl.

As shown in Table I, *Carcinides* dactyl chemoreceptors have limited sensitivity to many stimuli, which include various alcohols, sugars, acids, amino acids, and hypertonic sea water. They are moderately sensitive to a few amino acids and exhibit marked sensitivity only to l-glutamic acid among the substances tested. Although these receptors are somewhat responsive to acidity, that property of l-glutamic acid cannot be the primary source of its stimulatory ability since the dilution at effective concentrations is so great that there is a negligible pH change in sea water. At higher concentrations the receptors do not distinguish between neutralized and acid glutamic solutions. Further strong evidence of a high order of specificity in the glutamic response is the fact that all derivatives of glutamic acid which were available to us were no more active than glycine.

Of the three sets of optical isomers tested, those of glutamic acid and leucine were distinguished by preparations of only one to three units and all three by preparations containing many active units. In some instances (Fig. 2, A, B, C) the optical isomers of glutamic acid activate different units. We were unable to detect differences in the responses of single units to d- and l-aspartic acid although this does not preclude the existence of such differences because only a few such preparations have been tested. The characteristic responses to d- and l-leucine are illustrated in Figure 2, D–G, where d-leucine produces a considerably more prolonged burst than its antipode, although the initial phases of the responses to the two isomers are nearly identical in this instance.

In the light of the pronounced specificity which the dactyl chemoreceptors have been shown to have towards glutamic acid it becomes of interest to inquire into the nature of the active element of *Mytilus* extract. Amino acids are almost certainly involved since the extract does not lose potency upon boiling for five minutes and is dialyzable. Moreover, the same receptor population seems to be involved in the response to glutamic acid and to *Mytilus* extract since adapting the dactyl to either abolishes the response to the other.

FIGURE 2. Responses of *Carcinides* dactyl receptors to chemical stimulation. A, 5×10^{-3} *M* l-glutamic acid; B, 6×10^{-4} *M* l-glutamic acid; C, 3×10^{-2} *M* d-glutamic acid; D, 2.5×10^{-2} *M* d-leucine; E, same as D after elapse of 0.5 second; F, 2.5×10^{-2} *M* l-leucine; G, same as F after elapse of 0.5 second. A–C and D–G are from the same preparations. In A–C a larger mechanoreceptor unit is active and indicated by arrow to show chemoreceptor latency. Time base 100 mscs. between markers in A–C and the same throughout. Bar at lower right, 50 μV.

3. *Behavioral correlations*

In confirmation of Luther's observations, blinded *Carcinides* oriented readily to bits of mussel placed near any limb. Before the animal made the orienting movement, the nearest dactyl frequently probed the bit of tissue previous to making a precisely executed turn to present the tissue to the mouthparts. Inert materials were ignored. When *Mytilus* extract or .001 *M* l-glutamic acid was applied to the walking legs of crabs restrained on their backs in water shallow enough to expose all limbs, thus assuring stimulus localization, no sign of recognition could be seen. However, under the same conditions application of either substance to the chelae caused the beginning of feeding movements even before the stimulated claw was touched to the mouthparts. Touching the chelae of an unrestrained crab with either substance was followed by movement of the chelae to the mouth and chewing without orienting movements of the whole animal.

Discussion

The dactyl chemoreceptors of *Carcinides* are considerably more sensitive to amino acids than any arthropod preparation so far described and, indeed, approach in sensitivity the remarkable performance of insects responding behaviorally to sugars (see tabulation in Dethier and Chadwick, 1948, p. 244). Hence, when one considers that feeding responses were induced in *Carcinides* with pure solutions of glutamic acid, together with the high concentrations in animal tissues of glutamic acid, both free and bound (Meister, 1957), it becomes clearly possible that effective localization of food can be made simply on the basis of detection of low concentrations of amino acids.

The poor response of the dactyl preparation to reduced glutathione, known to be a highly specific inductor of feeding responses in at least two coelenterates (Loomis, 1955; Lenhoff and Schneiderman, 1959), reduces the possibility that there is a feeding-inducing substance common to large groups of animals, a suggestion which receives further support from the fact that the major feeding stimulus in the mosquito, *Culex pipiens,* is yet another compound, adenylic acid (Hosoi, 1959). It would therefore seem unwise to venture generalities concerning feeding-inducing substances except that they are likely to be of low molecular weight.

Since the observations of Piutti (1886) the optical isomers of a number of amino acids have been known to produce different tastes in man. The natural isomers may be tasteless, "meaty" in the case of glutamic acid, or bland, while d-amino acids commonly are sweet (Berg, 1953). Occasionally an isomer falls in one human taste modality and its antipode in another: l-leucine and l-isoleucine are bitter and their antipodes are sweet. These relationships, involving radical changes in taste upon subtle structural modification, have never been subjected to neurophysiological investigation despite the long time they have been known. The present findings appear to be the first to extend these observations to organisms other than man and are particularly significant in that they show at least some optical isomers of amino acids are distinguishable strictly in terms of the activity of small chemoreceptor populations. Despite the limited nature of this survey it is already apparent that at least three means exist by which these isomers can elicit differing receptor activity: they may simply activate different receptors, or have different threshold concentrations or adaptation times for the same receptors.

The molecular basis of these possible modes of action obviously cannot be discussed in the light of available direct experimental evidence. However, it is interesting to recall that metabolic systems acting upon d-amino acids are quite rare in multicellular organisms. To mention a pertinent example, active transport of amino acids across the mammalian intestine is specific to the l-isomers (Fridhandler and Quastel, 1955). Such observations lend support to various commonly invoked receptor activation schemes in which stimulant-receptor complexes are envisaged as involving formation on the basis of van der Waal's forces (see discussion by Dethier, 1956). But should more instances appear in which d-amino acids have more long lasting effects than their l-isomers, as appears to be the situation with d- and l-leucine, it may become necessary to consider the possibility of chemical processes functioning in receptor clearance, even though the behavior of the insect sugar receptor argues to the contrary.

Summary

1. Dactyl chemoreceptors were electrophysiologically demonstrated in *Carcinides maenas, Libinia emarginata, Callinectes sapidus,* and *Paguris pollicaris.*

2. In *Carcinides* the receptors respond to a boiled dialysate of *Mytilus,* and to l-glumatic acid at dilutions in excess of 5.0×10^{-5} *M.* Derivatives of glutamic acid and other amino acids are less stimulatory.

3. Optical isomers of glutamic acid, aspartic acid and leucine are distinguished by small populations of *Carcinides* dactyl receptors.

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